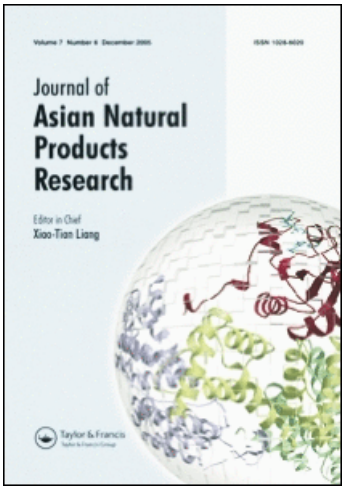

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Wen-Li Mei[a]; Ping Chen[a]; Hui Wang[a]; Jiu-Li Huang[a]; Hao-Fu Dai[a]
[a] Key Laboratory of Tropical Crop Biotechnology, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China

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ORIGINAL ARTICLE

Two new sesquiterpenes from endophytic fungus S49 of *Cephalotaxus hainanensis*

Wen-Li Mei, Ping Chen, Hui Wang, Jiu-Li Huang and Hao-Fu Dai*

Key Laboratory of Tropical Crop Biotechnology, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China

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Two new sesquiterpenes, 10,11,12-guaianetriol (**1**) and 1,10,11,12-guaianetetrol (**2**), were isolated from endophytic fungus S49 of *Cephalotaxus hainanensis* Li. Their structures were determined based on HR-ESI-MS and spectroscopic techniques (1D and 2D NMR).

Keywords: *Cephalotaxus hainanensis* Li; endophytic fungus; sesquiterpene; 10,11,12-guaianetriol; 1,10-guaianetetrol

1. Introduction

The cephalotaxine esters from the bark of *Cephalotaxus hainanensis* Li, such as harringtonine and homoharringtonine, have a good effect in the treatment of leukemia. Since *C. hainanensis* is the national secondary protected plant on the brink of extinction, much attention has been paid to look for an alternative source of these compounds other than the bark of *C. hainanensis* [1]. It is reported that endophytic fungi have developed the biochemical ability to produce compounds similar or identical to those produced by their host plants [2]. The report led us to start our investigation of the metabolites produced by endophytic fungi of *C. hainanensis*. In our previous study, 72 strains of endophytic fungi were isolated from the healthy bark, branches, and leaves of *C. hainanensis* tree collected in Jianfengling tropical rainforest reserve [3]. Some bioactive secondary metabolites have also been found from the endophytic fungi of *C. hainanensis* [4–7]. Further investigation on the secondary metabolites from the endophytic fungus S49 of *C. hainanensis* led to the isolation of two new sesquiterpenes, named 10,11,12-guaianetriol (**1**) and 1,10,11,12-guaianetetrol (**2**) (Figure 1), and their structures were unambiguously elucidated by HR-ESI-MS and extensive spectroscopic analysis (1D and 2D NMR). In this paper, we describe the isolation and identification of compounds **1** and **2**.

2. Results and discussion

Compound **1**, isolated as colorless oil, has a molecular formula of $C_{15}H_{27}O_6S^-$ based on its HR-ESI-MS (negative) at *m/z* 335.1519 M^-. The ^{13}C NMR and DEPT spectra of **1** presented 15 carbon signals for 3 methyls (δ 16.2, 20.3, 31.0), 6 methylenes (δ 23.2, 23.2, 26.1, 30.7, 33.7, 68.1) including one oxygenated carbon, 4

*Corresponding author. Email: hfdai2001@yahoo.com.cn

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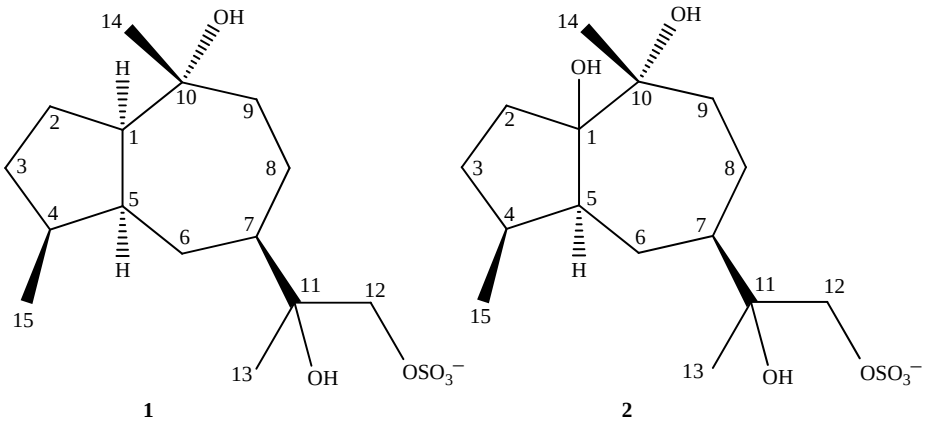

Figure 1. Structures of compounds **1** and **2**.

methines (δ 39.0, 44.1, 46.8, 55.4), and 2 oxygenated quaternary carbons (δ 74.8, 75.9). The corresponding proton signals were assigned from HMQC spectrum. The NMR spectral data of compound **1** were similar to those of a known guaianediol except for the appearance of an additional oxygenated methylene group at δ_C 68.1 in **1** and loss of a methyl group (C-12) in the guaianediol [8], which suggested that C-12 of compound **1** was substituted. The downfield chemical shift of C-12 (δ_C 68.1) indicated that the oxygenated methylene should be connected with a sulfate group [9,10]. This conjecture was further confirmed by the characteristic precipitate obtained with $BaCl_2$. Thus, the primary structure of **1** was obtained (Figure 1). The relative configuration at the chiral centers in compound **1** was supported by the ROESY spectrum (Figure 2). The NOE interactions from H-7 to H-1, H-4, H-5 showed that these four protons were at the same side. When they took α-orientation, CH_3-14 and CH_3-15 should be in β-orientation, which was supported by the NOE interactions from H-14 to H-15. The configuration of C-11 has not been determined in this study because of the low yield of compound **1**. Thus, the structure of compound **1** was elucidated and named as 10,11,12-guaianetriol.

Compound **2**, isolated as colorless oil, has a molecular formula of $C_{15}H_{27}O_7S^-$ based on its HR-ESI-MS (negative) at *m/z* 351.1472 M^-. The ^{13}C NMR and DEPT spectra of **2** presented 15 carbon signals for 3 methyls (δ 15.8, 20.1, 27.2), 6 methylenes (δ 23.3, 24.4, 30.1, 33.5, 34.1, 68.0) including one oxygenated carbon, 3 methines (δ 36.6, 43.5, 55.7), and 3 oxygenated quaternary carbons (δ 75.8, 76.8, 88.6), which were similar to those of **1**. The only obvious difference is the loss of a methine and the appearance of an oxygenated quaternary carbon at δ 88.6 in compound **2**. In the HMBC spectrum, the methyl proton signal at δ 1.26 (14-Me, s) was correlated with the carbon signals at δ 88.6, 76.8 (C-10, s), and 33.5 (C-9, t), so the oxygenated quaternary carbon at δ 88.6 was assigned to C-1 position. Thus, the primary structure of **2** was obtained and further confirmed by the analysis of HMQC, $^1H-^1H$ COSY, and HMBC spectra (Figure 2). The relative configuration at the chiral centers in compound **2** was identified by the ROESY spectrum similar to that of compound **1** (Figure 2). Based on the above evidence, the structure of compound **2** was elucidated and named 1,10,11,12-guaianetetrol.

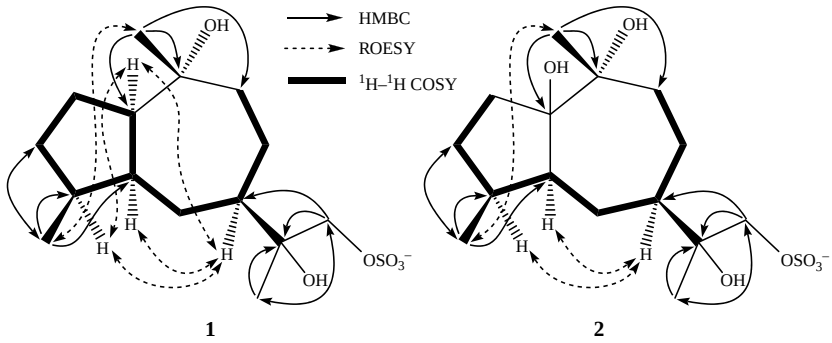

Figure 2. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of compounds **1** and **2**.

3. Experimental

3.1 General experimental procedures

Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rodolph Research Analytical, Hackettstown, NJ, USA). The IR spectra were obtained on a Nicolet 380 FT-IR instrument, as KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS spectra were measured with an API QSTAR Pulsar mass spectrometer. Column chromatographies were performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Fungal material

Endophytic fungus S49 was isolated from the bark of *C. hainanensis* tree collected in Jianfengling tropical rainforest reserve, Hainan Province, China (2005). This fungus has been deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, and maintained on potato dextrose agar (PDA) slant at 4°C.

3.3 Fermentation, extraction, and isolation

The endophytic fungus S49 was cultured on PDA at room temperature for 5 days. Three pieces of mycelial agar plugs ($0.5 \times 0.5\,cm^2$) were inoculated into 1 liter Erlenmeyer flasks containing 400 ml potato dextrose broth, then kept still at room temperature for 30 days. The culture broth (48 liters) was filtered to give the filtrate and mycelia.

The mycelia were extracted with 95% EtOH (2 liters) three times at room temperature. The extract was evaporated *in vacuo* to dryness and then partitioned in succession between H_2O and petroleum ether, EtOAc. The EtOAc solution was evaporated under reduced pressure to give a crude extract (3.0 g), which was separated into 10 fractions on a silica gel column using a step gradient elution of $CHCl_3$–MeOH (1:0–0:1, v/v). Fraction 6 (35.0 mg) was submitted to chromatography on silica gel column with petroleum ether–acetone (2:1, v/v) as the eluent, yielding compound **1** (10.5 mg).

The filtrate was evaporated *in vacuo* to a small volume and then partitioned in succession between H_2O and petroleum ether, EtOAc. The EtOAc solution was evaporated under reduced pressure to give a crude extract (6.0 g), which was separated into 15 fractions on a silica gel column using a step gradient elution of $CHCl_3$–MeOH (1:0 → 0:1). Fraction 11 (294.9 mg) was submitted to chromatography on a silica gel column with $CHCl_3$–MeOH (20:1) as the eluent and further separated by column chromatography over

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data for **1** and **2** (δ in ppm, J in Hz).

Position	**1** ($CDCl_3$)		**2** ($CDCl_3$)	
	δ_C	δ_H	δ_C	δ_H
1	55.4 (d)	2.05 (1H, m)	88.6 (s)	
2	26.1 (t)	1.71 (1H, m), 1.52 (1H, m)	34.1 (t)	1.95 (1H, m), 1.53 (1H, m)
3	30.7 (t)	1.74 (1H, m), 1.20 (1H, m)	30.1 (t)	1.90 (1H, m), 1.29 (1H, m)
4	39.0 (d)	1.99 (1H, m)	36.6 (d)	2.54 (1H, m)
5	46.8 (d)	1.91 (1H, m)	55.7 (d)	1.75 (1H, m)
6	23.2 (t)	1.92 (1H, m), 1.40 (1H, m)	23.3 (t)	1.94 (1H, m), 1.38 (1H, m)
7	44.1 (d)	1.88 (1H, m)	43.5 (d)	1.90 (1H, m)
8	23.2 (t)	1.40 (1H, m), 0.83 (1H, m)	24.4 (t)	1.78 (1H, m), 1.44 (1H, m)
9	33.7 (t)	1.94 (1H, m), 1.54 (1H, m)	33.5 (t)	1.83 (2H, m)
10	74.8 (s)		76.8 (s)	
11	75.9 (s)		75.8 (s)	
12	68.1 (t)	3.58 (1H, d, $J = 10.8$ Hz), 3.47 (1H, d, $J = 10.8$ Hz)	68.0 (t)	3.58 (1H, d, $J = 10.6$ Hz), 3.45 (1H, d, $J = 11.2$ Hz)
13	20.3 (q)	1.11 (3H, s)	20.1 (q)	1.07 (3H, s)
14	31.0 (q)	1.17 (3H, s)	27.2 (q)	1.26 (3H, s)
15	16.2 (q)	0.91 (3H, d, $J = 7.0$ Hz)	15.8 (q)	0.92 (3H, d, $J = 7.0$ Hz)

Sephadex LH-20 with 95% EtOH as the eluent, yielding compound **2** (4.0 mg).

3.3.1 10,11,12-Guaianetriol (*1*)

Colorless oil; $[\alpha]_D^{31}$ +35.2 ($c = 0.35$, $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}): 3397, 2950, 2855, 1374, 1045; ^{1}H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS (negative) *m/z*: 335.1519 M^- (calcd for $C_{15}H_{27}O_6S^-$, 335.1528).

3.3.2 1,10,11,12-Guaianetetrol (*2*)

Colorless oil; $[\alpha]_D^{31}$ +26.8 ($c = 0.50$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3468, 2948, 2837, 1372, 1050; ^{1}H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS (negative) *m/z*: 351.1472 M^- (calcd for $C_{15}H_{27}O_7S^-$, 351.1478).

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